American Journal of Hematology 64:67–70 (2000)

Drug-Induced Hemolysis: Cefotetan-Dependent Hemolytic Anemia Mimicking an Acute Intravascular Immune Transfusion Reaction

David Stroncek,[1]* Jo L. Procter,[1] and Judy Johnson[2]

[1]Department of Transfusion Medicine, Warren G. Magnuson Clinical Center, National Institutes of Health, Bethesda, Maryland
[2]St. Mary's Hospital, Richmond, Virginia

Numerous cases of drug-induced hemolytic anemia have been described in patients treated with penicillin or cephalosporin. Second and third generation cephalosporins are more commonly implicated in hemolytic reactions than first generation cephalosporins. We report a case of severe cefotetan-induced hemolytic anemia in a previously healthy 46-year-old woman undergoing an elective hysterectomy. The patient received 2 g of intravenous cefotetan intraoperatively and subsequently at 12 and 24 h post-operatively. She complained of diarrhea and fever on the third post-operative day and was seen in her gynecologist's office on the fifth post-operative day (hemoglobin = 10.5 g/dL). On the seventh post-operative day, she complained of fever and soreness around the suprapubic catheter site and was given a prescription for 500 mg oral cephalexin four times a day. The next day she was seen in the gynecologist's office and reported feeling better. Ten days after the operation her fatigue worsened and her hemoglobin was 4.8 g/dL. She was transfused with 3 units of packed red blood cells (PRBC) and was given 1 g of cefotetan intravenously. During the transfusion of the second unit of PRBC nursing staff observed gross hemoglobinuria and she subsequently developed acute renal failure. Laboratory chemistry parameters were consistent with severe acute hemolysis. The patient's direct antiglobulin test was reactive and her serum reacted with cefotetan-coated red blood cells (RBCs) and serum plus soluble cefotetan reacted with untreated RBCs. The titration endpoint of the serum against cefotetan-coated RBCs was 40,960, while the serum plus soluble cefotetan against uncoated RBCs was 2,560. This case of severe cefotetan-induced hemolysis was complicated by an acute hemolytic event that occurred during the transfusion of PRBC. Clinical and transfusion service staff must consider drug-induced hemolysis in the differential diagnosis of acute anemia. Am. J. Hematol. 64:67–70, 2000. Published 2000 Wiley-Liss, Inc.†

Key words: cefotetan; cephalosporins; penicillin; drug-dependent antibodies; hemolytic anemia

INTRODUCTION

Drug-dependent immune-mediated hemolysis is an uncommon, but often serious cause of hemolytic anemia that can result in renal failure, disseminated intravascular coagulation, and death. Approximately 3% to 12% of patients given penicillin or cephalosporin develop a positive direct antiglobulin test (DAT) [1]. When patients develop antibodies to drug bound to red blood cells (RBCs), there is the potential for hemolytic anemia. Reports of second and third generation cephalosporins causing drug-induced clinical hemolysis are increased as compared to first generation drugs [1]. Cefotetan is a second-generation cephalosporin with a wide spectrum of activity that is often used in surgical patients. We report a case of cefotetan-induced hemolysis due to a high titer drug-dependent IgG antibody that resulted in hemoglobinuria and acute renal failure. The clinical presentation and etiology of the hemoglobinuria was obscured by the concurrent administration of packed red blood cells (PRBCs).

*Correspondence to: David Stroncek, M.D., Department of Transfusion Medicine, 10 Center Drive, MSC-1184, Building 10, Room 1C711, Bethesda, MD 20892-1184.

Received for publication 25 June 1999; Accepted 5 January 2000

TABLE I. Patient Blood Counts and Blood Chemistries

Hospital day	Hemoglobin (mg/dL)	WBC ($\times 10^9$/L)	Total bilirubin (mg/dL)	LDH (U/L)	Creatinine (gm/dL)
Pre-surgery	14.0	7.0	N.T.[c]	700	0.7
Post-surgery[a]	9.3	4.9	N.T.	N.T.	N.T.
Out patient[b]	4.8	10.6	2.8	1,780	1.0
Day 1	6.5	16.9	20.8	10,923	1.4
Day 2	6.6	31.9	21.01[d]	24,678	3.7
Day 3	5.0	39.1	10.1	24,457	4.0
Day 4	6.9	26.1	4.3	11,177	4.2
Day 5	7.7	22.4	3.1	7,182	4.3
Day 6	6.6	24.7	2.9	5,992	4.2
Day 7	9.3	30.6	3.1	7,980	3.9
Day 8	7.8	25.1	1.5	4,358	4.2
Day 16	9.3	16.5	0.7	1,159	6.9
Day 19	12.1	12.5	N.T.	N.T.	N.T.

[a]Two days post hysterectomy.
[b]Immediately prior to admission.
[c]N.T., not tested.
[d]Direct bilirubin = 18.6 md/dL.

CASE REPORT

A transvaginal hysterectomy was performed on a 46-year-old white woman with three children and no prior blood transfusions. Her hemoglobin prior to surgery was 14.0 g/dL, she received no transfusions during the procedure, and following surgery her hemoglobin was 9.3 g/dL (Table I). She was given 2 g of intravenous cefotetan intraoperatively and again 12 and 24 h postoperatively. She was discharged 2 days after surgery, but on the third post-operative day she complained of diarrhea and fever. She was seen in the office of her gynecologist on the fifth post-operative day, at which point her hemoglobin was 10.5 g/dL and her white blood cell (WBC) count was 9.0×10^9/L. On the seventh post-operative day she complained of fever and soreness around the suprapubic catheter and was given a prescription for 500 mg oral cephalexin four times a day. On the eight post-operative day she reported feeling better and her temperature was 98.8°F. Ten days after the operation her fatigued worsened, her temperature was 100.8°F, and a vaginal cuff hematoma was suspected. Outpatient laboratory tests showed severe anemia and leukocytosis with a moderately elevated total bilirubin and lactate dehydrogenase (LDH) (Table I). Urine analysis was positive for blood, but no RBCs or WBCs were present. An abdominal ultrasound detected no hemorrhage. She was admitted to the hospital for antibiotic therapy and red cell transfusions.

On her first day in the hospital, one unit of PRBCs was transfused at 12:30 AM and 1 g of intravenous cefotetan was given at 2:30 AM. A second unit of PRBCs was transfused at 3:10 AM. During the transfusion of this unit, urine from the suprapubic catheter turned red and forty minutes later she developed chills and dyspnea. She was febrile, but her temperature did not differ from her pretreatment temperature. She was given a third unit of PRBC at 6:30 AM. At 9:30 AM a suspected transfusion reaction was reported to the hospital blood bank and her hemoglobin was 6.5 mg/dL, total bilirubin 20.8 mg/dL, and LDH 10,923 U/L.

She developed acute renal failure requiring dialysis. On the eighth hospital day her creatinine had risen to 4.2 mg/dL, her hemoglobin was 7.8 gm/dL, nucleated RBCs were noted on her blood smear, but her bilirubin had fallen to 1.5 mg/dL and LDH to 4,358 U/L. She left the hospital after 19 days still requiring dialysis, with hematologic and chemistry parameters reflecting resolution of hemolysis.

METHODS

Testing for Atypical RBC Antibodies

Serum was tested for atypical antibodies with three reagent screening RBCs and crossmatched with donor RBCs using an indirect antiglobluin test (IAT) [2]. The direct antiglobulin test (DAT) was performed on RBCs from EDTA-anticoagulated whole blood to determine if the RBCs were coated with IgG and/or C3d. Polyspecific antiglobulin reagent containing anti-IgG and anti-C3d, monospecific anti-IgG, and monospecific anti-C3d were run in parallel.

Testing for Drug-Dependent RBC Antibodies Using Drug in Solution

Equal parts of serum and drug in solution (1 mg drug per mL of phosphate buffered saline) were incubated at 37°C with reagent RBCs for 30 min. After incubation, the tubes were centrifuged at 3,500 rpm and examined for agglutination; reactants were washed 3 times with 0.9% saline and converted to an IAT using anti-IgG. Positive agglutination reactions were graded as 1+, 2+, 3+, or 4+. Testing was performed using cefotetan (Zeneca Pharmaceutical, Inc., Wilmington, DE), cephalexin (Biocraft Laboratories, Inc., Elmwood Park, NJ), and phosphate-buffered saline (Bio Whittaker, Walkersville, MD) at pH 7. A control consisting of equal parts of patient serum and PBS was tested in parallel with each drug solution.

Testing for Drug-Dependent RBC Antibodies Using Drug-Coated RBCs

For penicillin G (Marsam Pharmaceuticals Inc., Cherry Hill, NJ), 1 mL of washed and packed donor RBCs were incubated for 1 hr at room temperature in 40 mg (60,000 to 70,000 units/mL) of drug per mL of barbital-buffered saline at pH 9.6. For cefotetan and cephalexin. 1 mL of washed and packed RBCs were incubated for 1 hr at 37°C in 30 mg of drug per mL of PBS at pH 7. The drug-coated cells were washed 3 times and

TABLE II. Transfusion Reaction Investigation: Results of Serologic Testing on Patient Samples Collected Pre- and Post-Hemolytic Reaction

Assay	Sample	
	Pre-reaction	Post-reaction
Antibody Screen	37°C/Anti-IgG	37°C/Anti-IgG
Screening cells I, II, and III	Negative/negative	Negative/positive
DAT		
Anti-IgG	1+	3+
Anti-C3d	1+	Microscopic
Crossmatch	37°C/Anti-IgG	37°C/Anti-IgG
First unit transfused	Negative/negative	Negative/positive
Second unit transfused	Negative/negative	Negative/positive
Third unit transfused	Negative/negative	Negative/positive

resuspended in 0.9% saline. Serum was diluted 1:20 in PBS and tested against drug-coated RBCs in the IAT using tube and gel agglutination methods described above. As a control, uncoated donor RBCs from the same donor used to prepare drug-coated RBCs were tested in parallel.

RESULTS

Atypical RBC Antibodies

The patients blood group was O-positive, as were the 3 PRBCs units she received. Pre-reaction serum did not react with screening or donor RBCs by IAT. There was a panagglutinin present in the post-reaction sample that reacted with all screening and donor RBCs by IAT (Table II). The DAT performed on a sample collected before the patient's surgery was negative; IgG (1+) and complement (1+) were detected on a sample collected pre-transfusion: IgG (3+) and complement (microscopic) were detected on a sample collected post-reaction.

The DAT remained reactive and a weak panagglutinin was present in the patient's serum on day 11 of the hospitalization, and on day 16 her serum reacted weakly with 5 of 11 panel cells but without specificity for any of the clinically significant blood group antigens. On day 20 her serum reacted nonspecifically (2+) with 6 of 11 ficin-treated cells. These results are suggestive of the presence of a warm and/or a cold autoantibody.

Drug-Dependent RBC Antibodies

The patient's serum was reactive with cefotetan-coated RBCs after the 37°C (2+) incubation and the anti-IgG phase (4+) of the IAT. The serum was nonreactive with uncoated RBCs. A titration of the patient's serum against cefotetan-coated RBCs revealed an endpoint of 40,960 at the anti-IgG phase.

The patient's serum plus cefotetan in solution (1 mg/mL PBS) reacted after incubation at 37°C for 30 min (1+) and at the anti-IgG phase (4+). The patient's serum plus PBS did not react with reagent RBCs in either the 37°C or the anti-IgG phases of the assay. When patient serum was diluted in drug solution at 1 mg/mL concentration in PBS the titer of the cefotetan-dependent antibody was 2,560 at the anti-IgG phase.

The patient's serum and eluate prepared from her postreaction RBCs were tested against cephalexin-treated RBCs. The eluate reacted weakly with cephalexin-coated RBCs at the anti-IgG phase.

DISCUSSION

In five other cases of cefotetan-dependent hemolytic anemia reported between 1992 and 1994 there were three fatalities [3–7]. Our patient experienced acute renal failure and respiratory failure, but she recovered. The drug-dependent antibodies in one other case were high titer IgG antibodies [5]. In four cases, the antibody reacted with drug-coated RBCs [3–6] and in three cases the antibody also reacted with RBCs in the presence of soluble drug [3,5,6]. In this case report and two others there was evidence of a drug-independent antibody which reacted in the absence of drug [4,5]. A recent serologic study of antibodies in 43 patients with cefotetan-dependent immune hemolytic anemia found that sera in all of the patients contained antibodies that reacted with drug-coated RBCs, 98% contained antibodies that reacted with RBCs in the presence of soluble drug, and 44% of the sera had drug-independent antibodies [8]. These cases suggest that cefotetan-induced antibodies may react with RBCs to which drug is attached, with RBCs in the presence of the drug or with RBCs independent of drug.

In our patient, the brisk and severe cefotetan-induced hemolysis began almost immediately after her fourth infusion of drug given 10 days after her first three successive infusions. The drug-dependent hemolysis appeared to be a typical acute intravascular immune hemolytic transfusion reaction because PRBCs were transfused near the time when cefotetan was given. Given the IgG and complement were on her RBCs prior to the reaction and the blood type of the patient and PRBCs were the same, sources beyond the RBC transfusion were searched for the cause of the hemolysis. The detection of a high-titer antibody that reacted in the presence of cefotetan and with cefotetan-coated RBCs confirmed that the hemolytic event was drug-induced.

We suspect that this patient began producing an antibody to cefotetan after receiving 3 intravenous doses within 24 hr. When she was given cephalexin several days later, it is likely that the cefotetan-dependent antibody cross-reacted with the cephalexin and caused mild hemolysis. While taking cephalexin, she was anemic and had an increased LDH and a positive DAT, indicating that immune-mediated hemolysis occurred prior to the transfusion of RBCs and prior to the administration of the fourth intravenous dose of cefotetan.

For penicillin and cephalosporin-dependent antibodies that react with drug-coated RBCs the reactivity is usually quite strong. However, treating RBCs with drug is a laborious process that most transfusion services do not routinely perform. Patient samples must be referred to reference laboratories for a drug study. In this patient the cefotetan-dependent antibody was readily detected by simply adding equal parts of serum to drug solution and testing it against reagent RBCs using standard reagents and test procedures. The severity and uncertainties of this case illustrate the need to rapidly confirm a suspected diagnosis of drug-dependent hemolysis. A simple procedure for detecting drug-dependent antibodies using serum with soluble drug in the test and PBS in the control can be established in most transfusion service laboratories. When drug-dependent hemolysis is suspected, the assay should be performed without delay. However, the titer is lower when serum is tested with soluble drug and if the results are negative, testing should be repeated with drug-coated RBCs.

Drug-dependent hemolysis can be difficult to distinguish from other causes of hemolytic anemia, including transfusion reactions. Whenever hemolytic anemia occurs, a thorough drug history must be obtained and testing for drug-dependent antibodies should be performed immediately. The fact that the cefotetan-dependent antibody in this patient cross-reacted with cephalexin suggests that she should avoid exposure to cefotetan and cefotetan analogues or other cephalosporins.

REFERENCES

1. Garratty G. Immune cytopenia associated with antibiotics. Transfusion Medicine Rev 1993;4:255–267.
2. Vengelen-Tyler V, editor. Technical manual, 12th edition. American Association of Blood Banks: Bathesda, MD; 1996.
3. Gallagher NI, Schergen AK, Sokol-Anderson ML, Shenahan EJ, H Chaplin. Sever immune-mediated hemolytic anemia secondary to treatment with cefotetan. Transfusion 1992;32:266–268.
4. Chenoweth CE, Judd WJ, Steiner EA, Kauffman CA. Cefotetan-induced immune mediated hemolytic anemia. Clin Infect Dis 1992; 15:863–865.
5. Garratty G, Nance S, Lloyd M, Domen R. Fatal immune hemolytic anemia due to cefotetan. Transfusion 1992;32:269–271.
6. Peano GM, Menardi G, Quaranta L, Abello P, Landra M, Fenoglio S. A rapidly fatal case of immune haemolytic anaemia due to cefotetan. Vox Sang 1994;66:84–85.
7. Wagner BKJ, Heaton AH, Flink JR. Cefotetan disodium-induced hemolytic anemia. Ann Pharmacother 1992;26:199–200.
8. Arndt PA, Leger RM, Garratty G. Serology of antibodies to second- and third-generation cephalosporins associated with immune hemolytic anemia and/or positive direct antiglobulin tests. Transfusion 1999; 39:1239–1246.